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ANALGESIC ACTIVITY OF MARKETED POLYHERBAL FORMULATION

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ABSTRACT

Ayurveda is considered to be a form of complementary and alternative medicine (CAM) within the western world, where several of its methods such as herbs, massage, and Yoga as exercise or alternative medicine are applied on their own as a form of CAM treatment. *Herbalism* is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Herbalism is also known as botanical medicine, medical herbalism, herbal medicine, herbology, and phytotherapy. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts. Current scenario and the literature review highlight the undesirable effects of synthetic drugs and hence there is an upsurge in the development of herbal formulations. The present study was conducted with a view to evaluate the analgesic activity of the polyherbal formulation and compare with the standard synthetic drug available in the market. The major objective was also to assess the effective dose where in this formulation can act as a substitute to the synthetic drugs with lesser / no undesirable effects. From the results obtained, it can be concluded that 200mg/kg body weight showed the best analgesic activity and this activity was retained up to 60 minutes before declining. Thus the dose level further needs to be optimized using many more methods to assess analgesic activity.

Keywords: Polyherbal, Phytotherapy, Analgesic, Intragastric.

INTRODUCTION

Ayurveda is a system of traditional medicine native to India and practiced in other parts of the world as a form of alternative medicine. In Sanskrit, the word Ayurveda comprises the words *ayus*, meaning 'life' and *veda*, meaning 'science'. The Sushruta Samhita and the Charaka Samhita were influential works on traditional medicine during this era. Ayurvedic practitioners also claim to have identified a number of medicinal preparations and surgical procedures for curing various ailments and diseases. Ayurveda is considered to be a form of complementary and alternative medicine (CAM) within the western world, where several of its methods such as herbs, massage, and Yoga as exercise or alternative medicine are applied on their own as a form of CAM treatment. Changing civilization day by day by human beings has given birth to complication i.e. disease. When two or more herbs are used in formulations, they are known as polyherbal formulations. Sometime herbs are combined with mineral preparations also. The herbs often exist in crude state and Ayurveda describes method of purification of toxic herbs. The concept of polyherbalism is peculiar to Ayurveda although it is difficult to explain in term of modern parameters. Sanghar Samhita highlights the concept of synergism behind polyherbal formulations [1-3], An analgesic (also known as a painkiller) is any member of the diverse group of drugs used to relieve pain (achieve analgesia). The word analgesic derives from Greek an- ("without") and algos ("pain"). Analgesic drugs act in various ways on the peripheral and central nervous systems; they include paracetamol (acetaminophen), the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, narcotic drugs such as morphine, synthetic drugs with narcotic properties such as tramadol, and various others.“Pain is also defined as unpleasant sensation usually evoked by an external or internal noxious stimulation”.

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Ayurveda is the oldest healing system of medicine. Major formulations used in Ayurveda are based on herbs. The medicinal herbs are used as decoctions, infusions, tinctures, and powders.

The drug formulation in Ayurveda is based on two principles:

A. Use as single drug.

B. Use of more than two drugs.

When two or more herbs are used in formulations, they are known as polyherbal formulations. Sometime herbs are combined with mineral preparations also. The herbs often exist in crude state and Ayurveda describes method of purification of toxic herbs. The concept of polyherbalism is peculiar to Ayurveda although it is difficult to explain in term of modern parameters. Sanghar Samhita highlights the concept of synergism behind polyherbal formulations. Ayurveda has fundamental aspects for drug formulation. The herbs are selected according to the disease; other herbs are used to prevent the side effects arising from chief herb.

Analgesic

Before going to study the Analgesics we have to know what is the pain. Algesia (pain) is defined as a protective mechanism for the body, it occurs whenever any tissues are being damaged, and it cause the individual to react to remove pain stimulus. [(14)]

An analgesic (also known as a painkiller) is any member of the diverse group of drugs used to relieve pain (achieve *analgesia*). The word *analgesic* derives from Greek *an-* ("without") and *algos* ("pain").

Analgesic drugs act in various ways on the peripheral and central nervous systems; they include paracetamol (acetaminophen), the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, narcotic drugs such as morphine, synthetic drugs with narcotic properties such as tramadol, and various others.“Pain is also defined as unpleasant sensation usually evoked by an external or internal noxious stimulation”.

Types of Pain

From a temporary perspective, pain can be divided in acute and chronic. Acute pain occurs after traumas, operations, or a lesion of a nerve and pain is often recurrent. Chronic pain occurs continuously for at least 3 months. It inhibits feelings, emotions, thinking and reactions. Social interactions and work are restricted to the extent that mobility and physiological functions are inhibited. [(16)]

Nociceptive pain and neuropathic pain are the two main kinds of pain when the primary mechanism of production is considered. A third kind may be mentioned as psychogenic pain.

Damage to the nervous system itself, due to disease or trauma, may cause neuropathic or neurogenic pain. Neuropathic pain may refer to peripheral neuropathic pain, which is caused by damage to nerves, or to central neuropathic pain, which is caused by damage to the brain, brainstem, or spinal cord. Stimulation of a nociceptor, due to a chemical, thermal, or mechanical event that has the potential to damage body tissue, may cause nociceptive pain.

Nociceptive Pain

Nociceptive pain may be classified further in three types that have distinct organic origins and felt qualities. Superficial somatic pain (or cutaneous pain) is caused by injury to the skin or superficial tissues. Cutaneous nociceptors terminate just below the skin, and due to the high concentration of nerve endings, produce a sharp, well-defined, localized pain of short duration. Examples of injuries that produce cutaneous pain include minor wounds, and minor (first degree) burns.

Deep somatic pain originates from ligaments, tendons, bones, blood vessels, fasciae, and muscles. It is detected with somatic nociceptors. The scarcity of pain receptors in these areas produces a dull, aching, poorly-localized pain of longer duration than cutaneous pain; examples include sprains, broken bones, and myofascial pain.

Visceral pain originates from body's viscera, or organs. Visceral nociceptors are located within body organs and internal cavities. The even greater scarcity of nociceptors in these areas produces pain that is usually more aching or cramping and of a longer duration than somatic pain. Visceral pain may be well-localized, but often it is extremely difficult to localize, and several injuries to visceral tissue exhibit "referred" pain, where the sensation is localized to an area completely unrelated to the site of injury.

Neuropathic Pain

According to the most widely accepted definition, neuropathic pain is "initiated or caused by a primary lesion or dysfunction in the nervous system." Neuropathic pain cannot be explained by a single disease process or a single specific location of damage.

Neuropathic pain may be associated with abnormal sensations called dysesthesias which occur spontaneously and allodynias that occur in response to external stimuli. Neuropathic pain may have continuous and/or episodic (paroxysmal) components. The latter are likened to an electric shock. Common qualities of neuropathic pain include burning or coldness, "pins and needles" sensations, numbness and itching. Nociceptive pain is more commonly described as aching.

As much as 7% to 8% of the population is affected and in 5% it may be severe. Neuropathic pain may result from disorders of the peripheral nervous system or the central nervous system (brain and spinal cord). Thus, neuropathic pain may be divided into peripheral neuropathic pain, central neuropathic pain, or mixed (peripheral and central) neuropathic pain.

Central neuropathic pain is found in spinal cord injury, multiple sclerosis, and some strokes. Fibromyalgia, a disorder of chronic widespread pain, is potentially a central pain disorder and is responsive to medications that are effective for neuropathic pain.

Aside from diabetes (see Diabetic neuropathy) and other metabolic conditions, the common causes of painful peripheral neuropathies are herpes zoster infection, HIV-related neuropathies, nutritional deficiencies, toxins, remote manifestations of malignancies, genetic, and immune mediated disorders.

Neuropathic pain is common in cancer as a direct result of cancer on peripheral nerves (e.g., compression by a tumor), or as a side effect of chemotherapy, radiation injury or surgery [4,5]. The list of marketed analgesics are shown in table 1.

Pain Receptors and their Stimulation

The pain receptors in the skin and other tissue are all free nerve endings, they are widespread in superficial layer of the skin as well as in certain internal tissue such as periosteum, arterial wall and joint surface.

Analgesics are the drug that selectively relives pain by acting in the CNS or as peripheral pain acting mechanism, without significantly altering consciousness. Analgesics are mainly divided in to two groups.

- Opioid Analgesics/Narcotic Analgesics
- Non- Opioid/Non narcotic analgesics

Opioid Receptors

The Opioid analgesics are exerting their action by interacting with specific receptor present on neurons in CNS and peripheral tissue. The Radio-ligand binding studies have divide the Opioid receptor in to three subtypes i.e. μ, Kappa & δ.

- **μ-Receptors** It is characterized by its high affinity for morphine. Endogenous ligands for μ- receptor peptide called Endomorphins. Two subtypes of μ receptor have been proposed, they are
  μ1 → has higher affinity for morphine supraspinal analgesia and selectively blocked by naloxonazin
  μ2 → has lower affinity for morphine, mediates spinal analgesia, respiratory depression and constipating action.
- **κ (kappa)** It is characterized by its high affinity for ketocyclazocine and dynorphine A. Two subtypes of k-receptor k1 & k3 are functionally important.
- **δ (delta) receptor** : This receptor has high affinity for met/Leu Enkaphalins, which are its endogenous ligand peptide.

MATERIALS & METHODS

Chemicals and Reagents, Distilled Water**,** Corboxy methyl cellulose sodium (CMC) &Acetic Acid

Instruments / Apparatus: Eddy's Hot Plate, Stop watch & Syringe

Drugs Test drug: Herbal formulation X**,** Standard Drug: Aspirin [8-11]

PHARMACOLOGICAL ACTIVITY EVALUATION

Experimental Animals

Albino mice

Inbreed Albino mice of Wistar strain weighing in the range of 20 – 40 g were used for pharmacological activity evaluation.

Animal House Conditions

The animal house conditions maintained were: Temperature (22 ± 1) ^{0}C, Relative Humidity 65 ± 10 % and 12 hr (light) and 12 hr (dark) cycle. The animals were maintained in hygienic conditions in our animal house in 8 groups in clean plastic cages containing husk bed. The animals were fed with pelleted standard diet manufactured by Neutri Lab, Bangalore and drinking water *ad libitum.* The animals were allowed to acclimatize to our animal; house conditions for 6 to 7 days period prior to the experiment.

The institutional animal house is registered with CPCSEA and confirms the guidelines for the use and care of the experimental animal research. All experimental protocols involving animal studies were placed before the Institutional Animal Ethical Committee. The committee granted approval after carefully reviewing the protocol for the research project.

Evaluation of central and peripheral analgesic activity

***In Vivo* Models for Central Analgesic Activity**

Hot Plate Method

Principle

In this method heat is used as a source of pain. Animals are individually placed on a hot plate maintained at a constant temperature (55^{0}C) and the reaction of animals, such as paw licking or jump response is taken as the end-point. Analgesics increase the reaction time. The method was first described by Eddy and Leimbach (1953).

Groups

In this method male and female albino mice (20-40 Gm.) were used for the study. The animals were segregated into five groups of four animals each.

Group 1- Control (0.5% CMC suspension)
Group 2- Standard (Aspirin 200 mg/kg)
Group 3- Test drug (50 mg/kg)
Group 4- Test drug (100 mg/kg)
Group 5- Test drug (200 mg/kg)

The powder from the capsule were removed and formulated as a Suspension in 0.5% CMC. Suspensions were administered orally using intragastric tube at the dose described in procedure. The pain threshold (number of licking of paw/jumping) were measured at 30, 60, 90 and

120 minute after administration of standard and test solution by the use of hot plate maintained at 55^0C. A cut off time of 15 seconds was taken as a maximum analgesic response to avoid injury to the paws. The percent increase in reaction time in each time interval was calculated as percent inhibition.

Procedure

The mice were weighed and marked. Animals were divided into five groups as shown above, each consisting of 4 animals and appropriate volume of control, test and standard drugs were administered. After 30, 60, 90 and 120 minutes of the drug administration the reaction time in animals that is hind paw licking or jump response (whichever appears first) was observed and recorded on the hot plate maintained at constant temperature (55^0C). As the reaction time increases with test drug, 15 sec is taken as maximum analgesia and the animals are removed from the hot plate to avoid injury to paws.

Groups

In this method male and female albino mice (20-35 Gm.) were used for the study. The animals were segregated into five groups of four animals

Group 1- Control (0.5% CMC suspension)
Group 2- Standard (Aspirin 200 mg/kg)
Group 3- Test drug (50 mg/kg)
Group 4- Test drug (100 mg/kg)
Group 5- Test drug (200 mg/kg)

The powder from the capsule were removed and formulated as a Suspension in 0.5% CMC. Suspension were administered orally using intragastric tube. The method of Koster et al. was used. Suspension at dose described in procedure was orally administered to mice 1 hour before i.p. injection of 1% (v/v) acetic acid, at a dose of 10 ml/kg. 0.5% CMC was used as a control treatment while the reference group received 200 mg/kg of acetyl salicylic acid (ASA) as a standard. Writhings (a syndrome characterized by a wave of contraction of the abdominal musculature followed by extension of hind limbs) that occurred after acetic acid were counted for 10 minutes. A reduction in the writhing number as compared to the control group was considered as evidence for the analgesia, which was expressed as percent inhibition of writhing.

Statistical analysis

The results were subjected to statistical analysis using one way ANOVA Followed by "Bonferroni's Multiple Comparison Test" for both Hot plate method, and acetic acid induced writhing method. All the Data are presented as mean ± SD (Standard deviation) the P values < 0.001

RESULTS AND DISCUSSION

The analgesic activity of Polyherbal formulation was evaluated using both chemical and thermal methods. These methods were used to identify the effective oral dose

Calculate percent of increase in reaction time (as index of analgesia) at each interval.

Formula:

$$\% \text{ Pain Inhibition} = \frac{\text{Test} - \text{Control}}{\text{Test}} \times 100$$

***In Vivo* Models for Peripheral Analgesic Activity**

Acetic Acid induced method

Principle

Chemicals may pronounce painful reactions in animals also. Intraperitoneal injection of phenylquinone, bradykinin or acetic acid produces pain reaction, which is characterized as a writhing response. Constriction of abdomen, turning of trunk (twist) and extension of hind legs are taken as reactions to chemically induced pain. Analgesics, both narcotic and non-narcotic type, inhibit writhing response [12-15]

Procedure

The mice were weighed and marked.Animals were divided into five groups as shown above, each consisting of 4 animals and appropriate volume of control, test and standard drugs were administered. After 60 minutes appropriate volume of acetic acid solution (1% w/v) was administered to the all groups one by one and placed them individually under glass jar for observation. Note the onset on writhes. Record the number of abdominal contractions, trunk twist response and extension of hind limbs as well as the number of animals showing such response during a period of 10 min. Calculate the mean writhing scores in control, Aspirin, and treatment groups. Note the inhibition of pain response by aspirin and test drug.

Formula

$$\% \text{ Pain Inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

and maximum % pain inhibition for analgesic activity of Polyherbal formulation.

Eddy's Hot plate method Polyherbal formulation was administered to the mice at three different dose levels 50, 100 and 200mg/kg body weight. Paw licking and jumping on hot plate (whichever appeared first) was observed at 30, 60, 90 and 120 min for the all three doses.

Result When compared to standard, the Polyherbal formulation showed the results as described in table 2.

DISCUSSION

In Hot plate method experiment was done at three different doses 50, 100 and 200mg/kg body weight. Paw licking was observed at 30, 60, 90 and 120 min for the all three doses.

The maximum analgesic activity observed after 60 minutes where in there after the activity declined. (Graph 1)

Statistically by applying ANOVA followed by "Bonferroni's Multiple Comparison Test." The **c**ontrol group was compared with all the test dose level showed that 50 and 100mg/kg dose were nonsignificant and 200mg/kg dose was significant at the $P < 0.05$. Means it was effective in comparison with control.

LIST OF MARKETED HERBAL ANALGESICS [6-7]

Table 1. Marketed herbal analgesics

Product	Use and Company Name
Pain Massage oil	Pain massage oil is a herbal analgesic which provides relief from neuromuscular pain and the pain associated with arthritis. It is marketed by Himalaya pharmaceuticals.
Rumalaya forte tablet	Rumalaya forte tablets are used to relieve joint pain marketed by Himalaya pharmaceuticals.
Mahanarayan oil	Multi herb massage oil used in rheumatic pain, paralysis, gout, locked jaw, trisnus etc.It is marketed by Baidyanath pharmaceuticals.
Zandu Balm	A pain relieving rub that contains time-tested, active herbal ingredients. A gentle application of Zandu balm to the affected area brings the desired relief. Zandu Balm contains natural potent pain relievers for minor aches and pains from sprains, strains, backaches, arthritis, bruises and sports exertion. Oil of Gaultheria (Wintergreen) is widely used in medicine as external application for rheumatic disorders. It is the natural source of methyl salicylate which is a very effective analgesic for musculoskeletal disorders. It is marketed by Zandu pharmaceuticals.
Rhumasyl oil	Rhumasyl is a powerful and safe natural Ayurvedic rubefacient. Rhumasyl has prompt transdermal penetration and quick vasodilation and hence it is a potent and powerful muscle relevant. It is powerful and safe. Rhumasyl combines four most potent ingredients of natural oleation therapy for thenmatic disorders. Narayan tail in Rhumasyl Liniment & Ointment combines herbs which are anodyne, demulcent and emollient. Dashmoola (Ten roots) in the oil effectively tackle inflammation on all fronts. It is marketed by Zandu pharmaceuticals.

Table 2. Results of test drug by hot plate Method

Groups and Doses Given	Paw Licking response in Seconds at different time intervals in Mean ± SD			
	30 min.	**60 min.**	**90 min.**	**120 min.**
Control (0.5% CMC)	3.15 ± 0.111 ***	3.240 ± 0.080 ***	3.323 ± 0.091 ***	3.257 ± 0.136 ***
Aspirin 200mg/kg	6.643 ± 0.040 **(52.56%)**	8.58 ± 0.020 **(62.23%)**	10.94 ± 0.055 **(69.65%)**	12.08 ± 0.18 **(73.09%)**
Test drug 50 mg/kg	4.057 ± 0.186 **(22.22%) *****	4.59 ± 0.58 **(29.41%) *****	4.33 ± 0.26 **(23.32%) *****	3.72 ± 0.06 **(12.63%) *****
Test drug 100 mg/kg	5.133± 0.049 **(38.59%) *****	7.11 ± 0.023 **(54.43%)****	5.09 ± 0.15 **(34.77%)*****	4.10 ± 0.15 **(20.73%)*****
Test drug 200 mg/kg	7.163 ± 0.051 **(56%) ****	8.12 ± 0.14 **(60.09%) ^{ns}**	6.14 ± 0.11 **(45.92%) *****	4.85 ± 0.083 **(32.98%) *****

(Figures in bracket indicates % pain inhibition)

Each value is representing ± SD of four observations (n=4)

*P < 0.001, **P < 0.01, ***P < 0.05 Compared to standard.

Data was analyses by ANOVA followed by "Bonferroni's Multiple Comparison Test".

Table 3. Results of test drug by acetic acid induced writhing method

Groups	Number of writhings (10 min.) Mean ± SD)	% inhibition
Vehicle control (0.5%cmc)	34 ± 1.15***	--
Aspirin (Standard 200mg/kg)	10 ± 0.58	70.5 %
Test drug (50 mg/kg)	26 ± 1.15 ***	23.5 %
Test drug (100 mg/kg)	20 ± 1.53 ***	41.17%
Test drug (200 mg/kg)	15 ± 1.00 **	55.88 %

(SD-Standard Deviation)
Each Value is representing ± SD of Four observations (n=4).
*P < 0.001, **P < 0.01, ***P < 0.05 Compared to standard.
Data was analysed by ANOVA followed by "Bonferroni's Multiple Comparison Test".

Fig 1. Eddy's Hot Plate Method: No of Paw Licking/ Jumping Response and groups

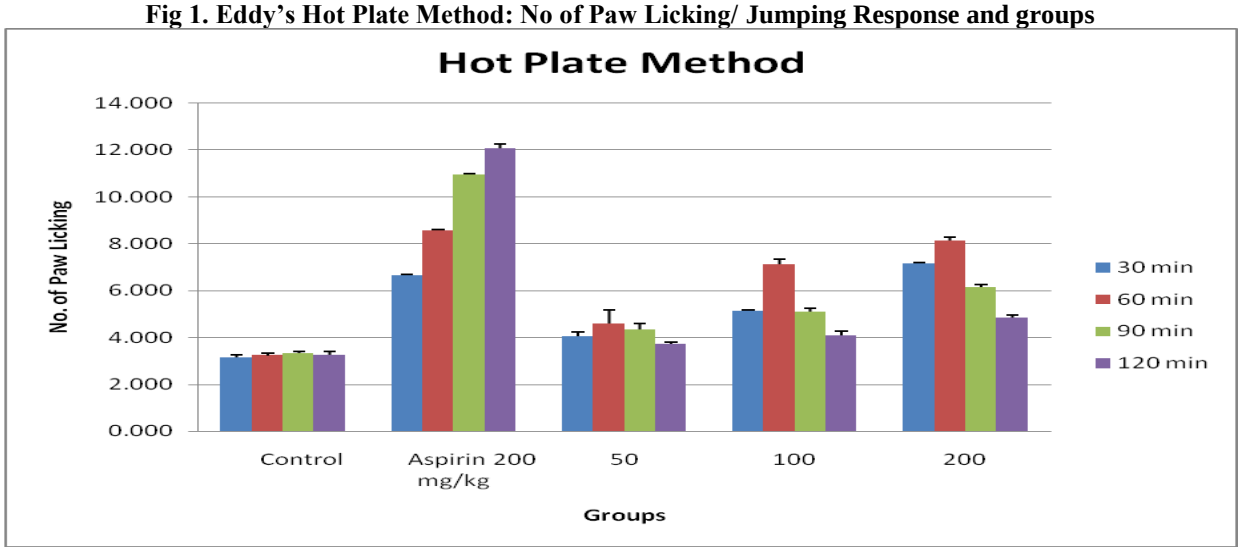
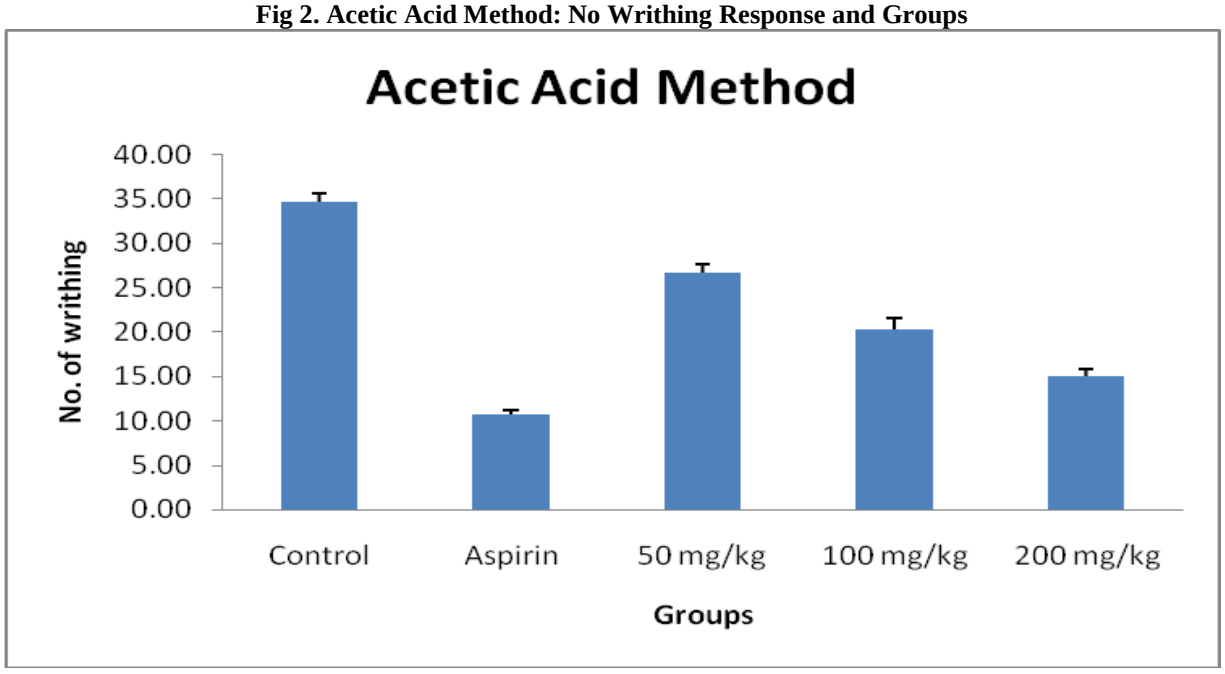


Fig 2. Acetic Acid Method: No Writhing Response and Groups



The Standard was also compared with all test groups which showed that 50 and 100mg/kg dose are significant and 200mg/kg dose is less significant with respect to Aspirin.

Acetic acid induced writhing method

Polyherbal formulation was administered to the mice at three different doses 50, 100 and 200mg/kg body weight and writhing (abdominal contractions) was observed by injecting acetic acid solution (1%w/v) 60 minutes after the administration. Record the number of abdominal contractions, trunk twist response and extension of hind limbs as well as the number of animals showing such response during a period of 10 minutes [16-18].

RESULTS

When compared to standard, Polyherbal formulation showed the results as described in table 3.

DISCUSSION

Experiment was done at three different doses 50, 100 and 200mg/kg body weight. All three doses significantly reduced the number of abdominal contractions and stretching of hind limbs induced by the injection of acetic acid in dose dependent manner. (Graph 2)

Polyherbal formulation 50, 100 and 200mg/kg exhibited a writhing inhibition percentage of 23.5%, 41.17% and 55.88% respectively. Statistically by applying ANOVA followed by "Bonferroni's Multiple Comparison Test." The control group was compared with all the test dose levels which revealed a significant difference in the pain inhibition/ threshold.

The standard was also compared with all test groups which projected that the standard aspirin showed the best activity as compared to all the three doses. When these three doses were compared amongst them, it could be said that there is a dose dependant response with respect to the analgesic [19-21].

CONCLUSION

Current scenario highlights the undesirable effects of synthetic drugs and hence there is an upsurge in the development of herbal formulations. The present study was conducted with a view to evaluate the analgesic activity of the Polyherbal formulation and compare with the standard synthetic drug available in the market. The major objective was also to assess the effective dose where in this formulation can act as a substitute to the synthetic drugs with lesser / no undesirable effects.

From the results obtained, it can be concluded that 200mg/kg body weight showed the best analgesic activity and this activity was retained up to 60 minutes before declining. Thus the dose level further needs to be optimized using many more methods to assess analgesic activity.

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